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ORIGINAL ARTICLE

Perturbation and delayed recovery of the reed invertebrate assemblage in Camargue marshes sprayed with *Bacillus thuringiensis israelensis*

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Abstract *Bacillus thuringiensis* var. *israelensis* (*Bti*) is the most commonly used larvicide to control mosquitoes worldwide. Considered as nontoxic to most organisms, *Bti* can nevertheless cause trophic perturbations to natural communities by reducing the abundance of Chironomidae, which are a key element of wetland food webs. Since August 2006, up to 8400 of the 33 000 ha of mosquito larval biotopes in the Camargue (Rhône delta, in southern France), are monitored by a public agency and *Bti*-sprayed (aqueous solution of VectoBac 12AS at 2.5 L/ha) whenever mosquito larvae (*Ochlerotatus caspius* and *Oc. detritus*) appeared in water bodies. This resulted in 30–50 aerial treatments/year, in addition to ground spraying of unknown frequency. The sprayed habitats include *Phragmites australis* reedbeds, which support a specific avifauna of conservation concern. We compared the abundance of invertebrate prey available to passerine birds at treated and control sites relative to the predicted values based on hydrology over a 9-year period. Food available to reed passerines was significantly reduced at treated areas, translating into a 34% decrease in breeding birds based on predictive modeling. The most affected arthropods were Diptera, Aranaea, Coleoptera, and Hymenoptera. No cumulative effects were observed over time, but the recovery of the invertebrate assemblage after the cessation of mosquito control was delayed due to *Bti* spore persistence and proliferation in the sediments. While hydrology remains a prime factor influencing primary and secondary productivity of the Camargue reed marshes, *Bti* spraying had significant negative effects on animal communities at several trophic levels.

Key words Camargue; insecticide impact; mosquito control; *Phragmites australis* marsh; trophic interactions; wetland conservation

Introduction

Thirty years after the discovery of its selective acute toxicity against mosquitoes (Goldberg & Margalit, 1977), *Bacillus thuringiensis* var. *israelensis* (*Bti*) became the most commonly used preparation worldwide; it is the only mosquito larvicide authorized in Europe (Lacey, 2007; Rowe *et al.*, 2008). This terrestrial bacterial agent is considered nontoxic to most aquatic organisms (Boivert & Boisvert, 2000; Lacey & Merritt, 2004), and direct effects on nontarget fauna are mostly limited to other Diptera (Nematocera), such as Chironomidae (Ali, 1981; Liber *et al.*, 1998; Stevens *et al.*, 2013) and Tipulidae (Oestergaarda *et al.*, 2006). Owing to their benthic habits, chironomids are likely to be exposed to *Bti* over periods much longer than water-filtering mosquitoes are (Dupont & Boisvert, 1985; Ohana *et al.*, 1987). Although chironomids are a key element of food webs in wetlands (Ali, 1995; Batzer & Wissinger, 1996), indirect effects of *Bti* spraying through food web perturbations have yet received little attention, accounting for only 4% of 311 studies on *Bti* environmental use according to a recent literature review (Poulin, 2012).

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A mosquito-control programme encompassing 8400 ha of the 33000 ha of potential larval biotopes in the Camargue (France) was launched in August 2006 to reduce the nuisance caused by *Ochlerotatus caspius* and *Oc. detritus* (Diptera: Culicidae) to the local human population and tourists. Studies of indirect effects on the nontarget fauna, based on the comparison of treated and control sites, were initiated in 2007 by independent research scientists under the aegis of the Parc Naturel Régional de Camargue. Among the habitats subject to mosquito control are marsh with common reed *Phragmites australis*, which cover 9200 ha in the Camargue (Davranche *et al.*, 2010). Reed marshes are the sole breeding habitat of 5 passerine species locally, including the resident Moustached warbler (*Acrocephalus melanopogon*) and Reed bunting (*Emberiza schoeniclus witherbyi*), both of conservation concern (Rocamora & Yeatman-Berthelot, 1999; Vera *et al.*, 2014). Reed marshes are structurally simple ecosystems well suited for modelling of ecological interactions (Mathevet *et al.*, 2003; Poulin *et al.*, 2010a; Lefebvre *et al.*, 2015). A study carried out in 1998–1999 showed that abundance of breeding passerines at 24 reedbed sites along the French Mediterranean coast (including the Camargue) was strongly correlated with that of their invertebrate prey, of which the relative abundance could be predicted based on the duration of flooding during the preceding year (Poulin *et al.*, 2002). Because hydrology is expected to have more impact on wetland ecosystems than *Bti* spraying (Batzer & Wissinger, 1996), we used this reference work (Poulin *et al.*, 2002) to address indirect effects of mosquito control on the trophic structure of the invertebrate community in Camargue reed marshes. More specifically, we tested whether the range of deviation between the observed and expected food availability index based on hydrology differs significantly between control and *Bti*-treated areas.

Materials and methods

Study area

The Camargue (the extensive delta of the river Rhône) in southern France is a mosaic of natural and human-modified wetland ecosystems spread over 100 000 ha of which 33 064 ha are potential mosquito-producing habitats (Fig. 1). To reduce the nuisance caused to humans, *Bti* spraying was initiated in the south-western (from August 2006) and south-eastern (April 2009

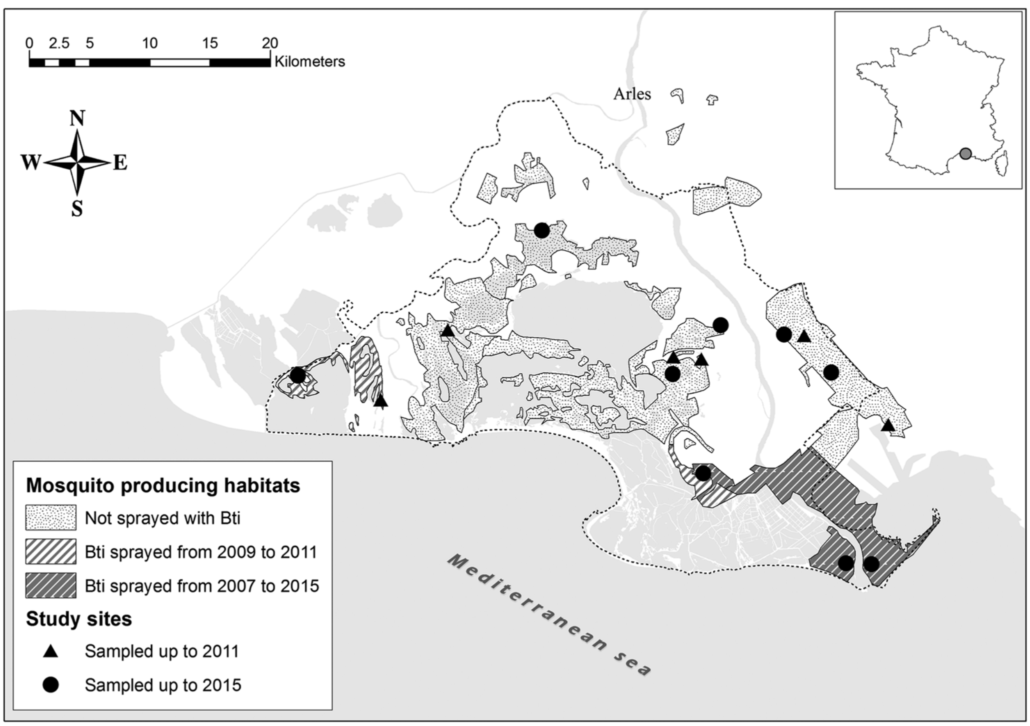

Fig. 1 The location of the study sites relative to mosquito-supporting habitats (estimated by EID-Mediterranee in 2005) and mosquito-controlled areas within the limits of the Regional Natural Park of the Camargue.

to October 2011) parts of the delta by the Entente Interdépartementale de la Démoustication (EID), a public agency in charge of mosquito-control operations in France (www.eid-med.org/). Mosquito control consists of aerial or ground spraying of an aqueous solution of VectoBac 12AS® at 2.5 L/ha whenever *Ochlerotatus caspius* and *Oc. detritus* larvae appear in water bodies that are regularly monitored by EID technicians. The frequency of *Bti* spraying depends on rainfall and water management that trigger mosquito larval development; in any year the same water body can be sprayed several times. Overall, 30–50 aerial treatments are carried out yearly, representing a cumulated sprayed area of 5000–8000 ha in the south-western area, in addition to ground spraying, which accounts for 32% of the sprayed areas (EID-Méditerranée, 2015). No specific information is available on the studied reed marshes in terms of number of *Bti* treatments per year, but the mean density of *Bti* spores (see Duchet *et al.*, 2014 for methodological details) differed significantly ($F_{1,502} = 87.4$, $P < 0.0001$) between control (1916 spores/g of soil) and treated (86 761 spores/g of soil) sites (Poulin, Lefebvre & Després, unpublished data).

Field sampling

We monitored up to 5 treated sites and 10 control sites between 2007 and 2015. The number of sites monitored each year depended on the sprayed areas, site accessibility and funding (Table 1). Interruption of mosquito-control operations at 1 of the 2 sites located in the south-eastern part of the delta allowed us to observe the community recovery process over 4 consecutive breeding seasons after the last *Bti* spraying in late autumn 2011.

Our sampling scheme was a replicate of the reference study carried out by Poulin *et al.* (2002). At each study site, water levels were measured monthly using a PVC tube buried 50 cm into the ground within the marsh. Invertebrates were sweep-netted once a year during the bird breeding season in late May or early June. Reed vegetation was sampled with 500 sweeps using a 28-cm diameter insect net under windless and dry conditions in late morning or early afternoon (when the vegetation was dry). Captured invertebrates were identified to taxonomic order, counted and assigned to 1 of 5 size classes (0–2.5, 2.6–5, 5.1–7.5, 7.6–10, and >10 mm) based on body length.

Modelled hydrology–invertebrate–bird relationships

Using the number of sweep-netted invertebrates, we calculated a food availability index using the Equation (1) (Poulin & Lefebvre, 1997):

$$\sum_{i=1}^{n} p_i \frac{x_{ij}}{y_i}, \qquad (1)$$

where x_{ij} is the number of arthropods from group i (taxon and size) sweep-netted on site$_j$, y_i is the number of invertebrates from group i collected on all sites, and p_i is the proportion of invertebrates from group i found in the bird diet according to Poulin *et al.* (2002). Forty-seven groups of invertebrate prey combining 16 taxonomic categories and 5 size classes were used to calculate this index, which provides a relative value, useful for multisite comparisons (Poulin & Lefebvre, 1997). In reed marshes of southern France, the index of food availability (IFA) was positively correlated with the number of mist-netted passerines ($r = 0.62$, $P = 0.002$, $n = 22$) according to the regression Equation (2) (Poulin *et al.*, 2002):

$$Bird\ abundance = 16.4 + 0.063 \times IFA. \qquad (2)$$

This model allows us to estimate to which extent a reduction in food availability would affect passerine abundance in reed marshes.

The index of food availability in reed marsh is inversely related to the duration of ground dryness in the second part of the preceding year ($r = -0.74$, $P < 0.001$, $n = 22$, Poulin *et al.*, 2002). A theoretical food availability index (TIFA) was then calculated for each reedbed site and sampling year using the empirical Equation (3):

$$TIFA = 611 - 65n, \qquad (3)$$

where n is the number of months with dry ground between June and December the year before invertebrate sampling in the following spring.

Table 1 Number of examined reed marshes in the Camargue, southern France, from 2007 to 2015 relative to spraying.

	2007	2008	2009	2010	2011	2012	2013	2014	2015
Control sites	10	10	10	10	10	5	5	5	5
Bti-treated sites	2	3	5	5	3	3	3	3	3
Formerly *Bti*-treated site	0	0	0	0	0	1	1	1	1

Statistical analyses

The observed and predicted indices of food available to passerines were compared with a Generalized Linear Model using a nested-ANOVA design with year and site nested in treatment (*Bti* spraying). This method is perfectly adapted to experimental designs using pseudoreplicates (Sokal & Rolhf, 1995), and is considered as an excellent alternative to repeated-measure analyses in the case of variation among experimental units or missing data (Davis, 2002). The same statistical approach was used to compare the number of invertebrates captured from each taxon at treated versus control sites. Invertebrate abundance was corrected for hydrology and log-transformed. Bonferroni corrections were applied to take account for the multiple tests.

Results

Food availability index

Overall, the mean IFA was −19 at control sites and −281 at treated sites, relative to the predicted values based on hydrology (Fig. 2). This difference was significant ($F_{3,66} = 16.4$, $P < 0.0001$), and would correspond to a 34% decrease in bird abundance at treated sites. *Bti* spraying contributed to 82% of the explained variance in IFA compared to 12% for site and 5% for year.

The site where mosquito-control was interrupted after 2011, still had a high density of *Bti* spores in the soil in 2012 (mean of 500 083 spores/g of soil), which probably explains the low IFA value observed until 2013 (Fig. 2). By 2014, IFA increased sharply, in parallel with a sharp decrease in *Bti* spore density (2471 spores/g of soil). IFA at that site did not differ significantly from that of treated sites in 2012–2013 (Scheffé *post hoc* test, $P = 0.84$), being similar to that of control sites in 2014–2015 (Scheffé *post hoc* test, $P = 0.22$).

We tested *a posteriori* the relationship between food availability and hydrology at the treated versus control sites using ANCOVAs with year as covariable. There was a significant relationship between food availability and ground dryness in the preceding year at control sites ($F_{1,67} = 6.0$, $P = 0.016$) similarly to what was reported in the reference study, but not at treated sites ($F_{1,20} = 0.01$, $P = 0.91$).

Invertebrate abundance

Sixteen invertebrate taxa were sampled with sweep net, 5 of which were found in more than 95% of the samples (Table 2). All taxa occurring in at least 10% of the samples showed a lower abundance at treated sites, often representing a 50% reduction (Table 2). However, due to the high variability in the number of captures among sites and years, differences were significant for Diptera, Aranea, Coleoptera, and Hymenoptera (ants) only.

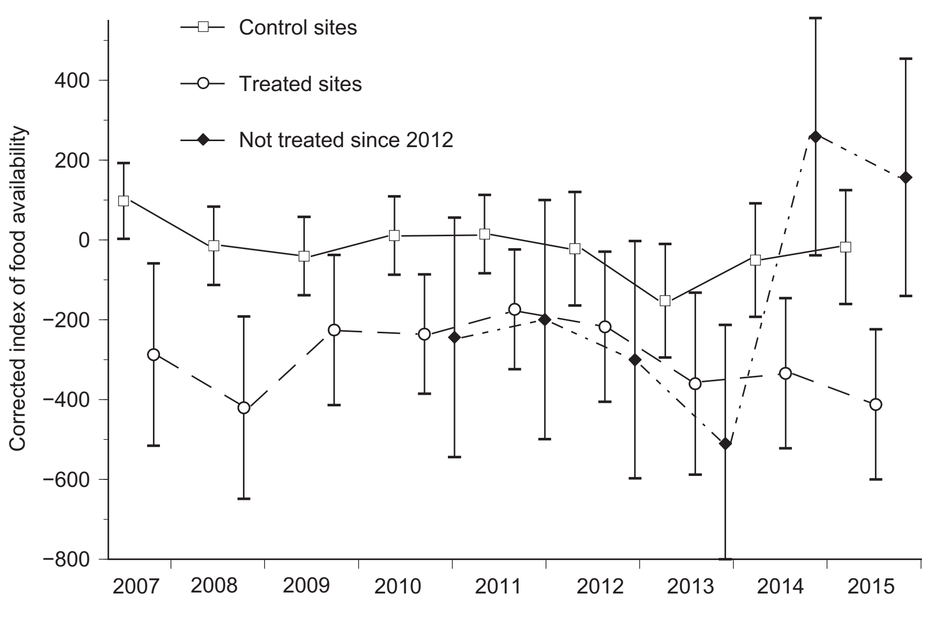

Fig. 2 Difference between the observed and predicted values based on hydrology of the mean food availability indices (with 95% CI) at treated and control sites.

Table 2 Abundance (mean numbers of individuals ± 95% CI) of each taxon on all sites sweep-netted since 2007 with results from ANOVA and percent occurrence in samples.

Taxa	Control sites	Treated sites	$F_{1,89}$	P	P corrected	Occurrence, % of samples
Diptera	201.3 ± 48.6	85.4 ± 75.3	9.07	0.003	*	99
Homoptera	88.26 ± 25.7	41.3 ± 39.8	1.52	0.221		97
Hymenoptera (wasps)	64.9 ± 14.3	31.0 ± 22.2	4.45	0.038		98
Coleoptera	38.9 ± 7.93	14.1 ± 12.3	20.04	< 0.001	**	96
Thysanoptera	33.4 ± 11.2	14.9 ± 17.4	0.11	0.746		83
Araneae	26.5 ± 5.0	11.0 ± 7.7	11.94	0.001	*	96
Pseudoscorpionidae	15.6 ± 8.4	2.3 ± 12.6	1.85	0.177		27
Heteroptera	7.4 ± 2.5	4.6 ± 3.9	0.07	0.789		59
Hymenoptera (ants)	5.6 ± 2.3	0.5 ± 3.6	9.39	0.003	*	43
Insecta (egg, pupae, larvae)	5.5 ± 3.1	3.1 ± 3.4	0.922	0.339		71
Acarina	3.0 ± 2.5	2.1 ± 3.9	0.04	0.833		31
Gastropoda	2.7 ± 1.6	0.5 ± 2.5	3.37	0.070		34
Odonata	1.5 ± 0.7	0.5 ± 1.2	1.98	0.164		30
Lepidoptera	0.4 ± 0.5	0.0 ± 0.8	0.22	0.643		9
Orthoptera	0.3 ± 0.2	0.2 ± 0.4	2.18	0.143		21
Neuroptera	0.1 ± 0.5	0.5 ± 0.5	0.10	0.756		7

Note: $^{*}P < 0.05$, $^{**}P < 0.01$. Bonferroni corrections for multiple comparisons were used.

Discussion

This study indicated a sustained and general impoverishment of the reed invertebrate fauna from the first year after the initiation of *Bti* spraying, significantly reducing food levels for passerines during the breeding season in Camargue reed marshes. Moreover, the absence of relationships between flooding duration and food abundance index at mosquito-controlled sites, suggest that *Bti* effects are strong enough to buffer the impact of hydrology on invertebrate abundance in reed marshes. We did not detect cumulative effects of *Bti* use over time, even at treated sites that were monitored during the 9-year study period. However, persistence and proliferation of *Bti* spores at the surface of sediments caused a 2-year delay in the recovery of the invertebrate assemblage at 1 site where mosquito control was interrupted. This phenomenon of *Bti* persistence and proliferation had previously been observed under certain environmental conditions (Tilquin *et al.*, 2008; Tétreau *et al.*, 2012; Duchet *et al.*, 2014). We suspect that the sharp decrease in *Bti* spore density 2 years after the interruption of spraying was related to the drying of the marsh.

Bti significantly reduced the abundance of dipterans, but also that of their potential predators such as spiders and beetles, which altogether accounted for 56% of food items in the diet of reed marsh-breeding passerines (Poulin *et al.*, 2002). The significant reduction of ants at treated sites could be the result of a higher exploitation of ants as substitute prey by birds in response to the reduction in the abundance of *Bti*-sensitive arthropods. A similar pattern was observed with House martin (*Delichon urbicum*) colonies nesting in *Bti*-treated areas in the Camargue, where the lower intake of Diptera, Araneae, Odonata and Neuroptera by nestlings was compensated by a higher intake of flying ants (Poulin *et al.*, 2010b; Poulin, 2012). This diet modification translated into a reduced breeding success (33% lower chick survival) due to the low food value of hard-cuticled ants, especially for the early nestling stages.

While studies of *Bti* toxicity on nontarget invertebrates are relatively numerous (summarized in Boisvert & Boisvert, 2000; Lacey & Merritt, 2004), indirect effects of *Bti* through food web perturbations have received little attention (reviewed in Poulin, 2012). About a third of these few studies (Purcell, 1981; Wipfli & Merritt, 1994; Hershey *et al.*, 1998; Jakob & Poulin, 2016) documented significant effects on abundance or diet of nontarget invertebrate species. Purcell (1981) observed a reduced abundance of backswimmers (*Notonecta indica*) in salt marshes sprayed with *Bti*. Hershey *et al.* (1998) showed a 57%–83% reduction in insect abundance and a 50%–83% decrease in biomass in the second and third year after initiation of *Bti* spraying based on the comparison of

18 wetlands, half of which were *Bti*-sprayed. Wipfli & Merritt (1994) observed different dietary response by Plecoptera and Odonata species following a reduction of black fly densities in streams, depending upon their diet specialization and black fly abundance in the environment. Jakob & Poulin (2016) observed a 50% reduction in richness and abundance of adult Odonata monitored along 6 transects during 5 years in the Camargue. While several factors can explain the lack of significant effects or their detection (reviewed in Poulin, 2012), resource shrinkage caused by mosquito control is expected to be disproportionally large in environments such as the Camargue where *Bti*-sensitive insects are particularly abundant, favouring their selection as preferred prey by the nontarget fauna (Murdock, 1969; Hansen & Beauchamp, 2014). Intensive spraying of *Bti* in Camargue reedbeds caused important collateral damage to the nontarget fauna, calling for precautionary use in natural protected areas. Efficiency of alternative techniques to reduce mosquito nuisance adapted to the specific context of the Camargue (i.e., small villages surrounded by wetlands covering large areas) are currently being tested. Among the most promising option are mosquito traps used as a protecting belt around inhabited areas.

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Disclosure

The authors declare no conflict of interest.

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